Part C

Hazard Assessment and Review of Available Studies

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Part B of this document described a variety of experiments demonstrating an effect of atrazine on the pituitary-hypothalamic-ovarian axis of the rodent. These alterations would be expected to result in the establishment of a hormonal environment conducive to the development of mammary and pituitary tumors (*i.e*., prolonged exposure to endogenous serum estrogens and serum prolactin).These neuroendocrine perturbations might also be expected to have effects other than tumorgenicity.

The suppression of LH release from the pituitary (described in Part B, section 9.2.7) might be expected to affect pregnancy in the rat. Alterations in LH release from the anterior pituitary might also be expected to affect pubertal development. Atrazine exposure has also been shown to alter serum prolactin levels following acute, high-dose exposure (Cooper *et al.*, 2000; these data are not discussed in Part B). Alterations of serum prolactin could affect pregnancy and pubertal development as well as having other potentially adverse effects -- specifically an increased risk of prostate inflammation in adults male rats whose mothers were exposed to atrazine while nursing.

Data describing atrazine effects on serum prolactin and possible effects of LH suppression and serum prolactin alterations on pregnancy, pubertal development, and prostatitis are described below. Most of these data are derived from the labs of Dr. Ralph Cooper at EPA. Also described is a study by Dr. Barry Zirkin of Johns Hopkins University examining atrazine effects on pubertal development in male rats. Understanding the data described below requires some background knowledge of the role LH and prolactin play in pregnancy and pubertal development in the rodent.

Brief backgrounds of the roles of prolactin and LH in pregnancy and pubertal development, followed by a discussion of the data showing atrazine's effects on these parameters, constitute the bulk of this chapter. The remainder of Part C is a brief discussion of a trio of open literature epidemiology studies that investigate potential associations of reproductive anomalies with atrazine exposure, and a discussion of a trio of studies from the lab of Dr. Jasna Kniwald in Yugoslavia that examine possible effects of atrazine on testosterone metabolism in the male rat.

The discussion of the testosterone metabolism studies immediately follows. A discussion of the epidemiology studies is next followed by a discussion of atrazine's effects on pregnancy, puberty and prostatitis.

10. Possible Effects of Atrazine Exposure on Testosterone Metabolism

The steroid hormone testosterone is converted into 5á-reduced metabolites by tissues *in vivo*. These 5á-reduced metabolites are primarily 5á-dihydrotestostrone (DHT), 3á-diol and androstenedione. Tissues such as the prostate, seminal vesicles, hypothalamus and pituitary contain 5á-reductase enzymes and are primarily responsible for reducing testosterone to these compounds. The enzymes responsible for these conversions are 5á-reductase, 3á-HSD and 17â-HSD. The 5á-reduced metabolites (particularly DHT) are able to bind the testosterone receptor, and testosterone receptor-5á-reduced metabolite complexes are believed to be more stable than testosterone-receptor complexes. The rat prostate also has receptors specific for DHT.

Three published papers have examined the effect of atrazine on testosterone metabolism. The first of these papers examined the effects of atrazine exposure with either *in utero* or *in utero* plus early postnatal exposure (Kniewald *et al.*, 1987). Female Fischer-344 rats were treated by s.c. injection once a day during their entire pregnancy with 16.6 mg/kg/day of atrazine or deethyatrazine (DET) and sacrificed at PND 28; or were treated once daily by s.c. injection with the same dose of both compounds during both pregnancy and lactation and were sacrificed at 21 days postnatal. Activity of pituitary reductase enzymes, and DHT and estradiol receptor binding sites in the prostate and uterus were determined in all animals.

A second study exposed young adult male Fischer-344 rats to either 60 or 120 mg/kg/day atrazine or DET by gavage for seven days (Babic-Gojmerac *et al.*, 1989). On the eighth day animals were sacrificed, and the anterior pituitary and hypothalamus were excised and activity of reductase enzymes responsible for testosterone metabolism were measured. *In vitro* studies were also conducted in which the anterior pituitary and hypothalamus were removed from young adult males and exposed to atrazine and DET *in vitro*. The activity of reductase enzymes was then determined.

The third study also used young-adult male Fischer-344 rats and dosed them by gavage with 120 mg/kg/day of atrazine for seven days (Simic *et al.*, 1991). On the eighth day the animals were sacrificed and DHT-receptor complexes in the prostate were measured.

These studies indicate that testosterone metabolism is not greatly altered if animals are treated only *in utero*. Testosterone metabolism can be significantly altered if male and females are treated *in utero* and during lactation or if males are treated as young adults. The studies indicate that atrazine and DET are able to inhibit the conversion of testosterone to its more active reduced forms. The inhibition of metabolism of testosterone to its more active forms by atrazine indicates an anti-androgenic effect of atrazine which may result in adverse consequences.

11. Epidemiology

11.1 IUGR in Iowa Communities

The incidence of intrauterine growth retardation (IUGR) in Iowa was investigated in an ecologic study (Munger *et al.*, 1997). The definition of IUGR can vary considerably (Seeds, 1984). IUGR can, generally speaking, be defined as low birth weight. Infants that are delivered full-term are usually defined as displaying IUGR if they weigh less than 2,500 grams at birth. For infants that are delivered prior to full term, birth weight below the tenth percentile for gestational age is frequently used to define IUGR.

IUGR was defined in the Munger study as birth weight below the 10^{th} percentile for gestational age as defined by California standards for non-Hispanic whites. The Munger study was a an ecologic study in which estimated exposures to pesticides through drinking water were compared to birth weights in an attempt to determine associations between the two. Data on levels of pesticide contamination in drinking water were obtained from the 1986 to 1987 statewide municipal water survey of Iowa which included data from 856 municipal water sources across the state. Birth weight data were obtained from birth certificate data obtained from the Iowa Department of Public Health. Factors such as maternal smoking, maternal education, quality of prenatal care, geographic region and community size were evaluated in an attempt to account for potentially confounding data.

An association between IUGR and maternal exposure to drinking water from the Rathburn system was found. The Rathburn system is a community drinking water system that obtains its water from the Rathburn reservoir in southern Iowa and serves several communities and counties in southern Iowa. The 1986 to 1987 drinking water survey found the Rathburn system to have elevated levels of herbicide contamination with the most notable contaminant being atrazine. The mean atrazine contaminant level in the Rathburn system 2.2 µg/L compared to a mean atrazine contaminant level of 0.6 µg/L in all other Iowa surface water suppliers. The rate of IUGR incidence from 1984 to 1990 in communities served by the Rathburn system was 11.2% compared to 6.4% for all other surface water suppliers in Iowa.

Regression models of IUGR showed that atrazine had the best fit (*i.e.*, the best positive association between drinking water contaminant levels and IUGR incidence) of all the contaminants examined in the study. The study authors note: "Atrazine had the best fit in the regression models of IUGR, but independent effects of other herbicides, which are intercorrelated, cannot be ruled out."

Although useful, the Munger study should be, because it is an ecologic study, regarded as a preliminary study which needs to be verified by more detailed epidemiologic studies. The deficiencies of ecologic studies are discussed in Part B section 4.9 and the study authors of the Munger study state:

> "Because of the limitations of the ecologic design of this study, including aggregate rather than individual measures of exposure and limited ability to control for confounding factors related to source of drinking water and risk of IUGR, a causal relationship between any specific water contaminant and risk of IUGR cannot be inferred."

11.2 Birth defects in Rural Minnesota

A study published in the open literature examined the possibility that offspring of pesticide appliers may display higher incidences of birth defects (Garry *et al.*, 1996). This study used information from the Minnesota Department of Agriculture (MDA) to identify persons in Minnesota who were certified to apply restricted use pesticides in 1991. This applicator information was linked to birth data supplied by the Minnesota Center for Health Statistics of the Minnesota Department of Health to determine birth defect rates among pesticide applicators in Minnesota. The birth data were also compared to pesticide use data supplied by MDA to examine associations between birth defects and quantitative pesticide use for both pesticide applicators and the general population.

Minnesota pesticide applicators did have significantly ($p<0.001$) more children born with a birth anomaly than did the general population of Minnesota. However, when birth data from pesticide applicators from one of three crop growing regions are compared to birth data from the general population from the same region, a statistically-significant difference ($p<0.02$) is seen only in one of the three regions. Use of any specific pesticide was not implicated in the increases in birth defects seen in pesticide appliers *vs.* the general population.

When use of specific pesticides was examined by comparing pesticide use by county cluster to birth defects in that cluster, the authors noted that use of chlorophenoxy herbicide and fungicides seemed to have the strongest association with birth defects. The results for atrazine use did not show a clear association of atrazine use with birth anomalies. There was a significant increase in birth defects when counties with atrazine use levels of >100,000 lbs AI/county cluster are compared to county clusters with <100,000 lbs AI, but if clusters with use levels of >25,000 lbs AI are compared to clusters with <25,000 lbs AI/cluster then a significant association is not seen.

11.3 Male Pesticide Exposure and Pregnancy Outcome

To examine the potential effects of paternal pesticide exposure on pregnancy outcome, farm activity in male farmers in Ontario, Canada was evaluated for the three months prior to conception through conception (Savitz *et al.*, 1997). Male farmers were surveyed about their farming activities for the past five years to obtain information about their activities during the above-described time around conception. Pregnancy outcomes were determined by questionnaires completed by the farm couples. Farm activities by the males were compared to pregnancy outcome to determine potential relationships of farm activities (specifically mixing, loading or applying pesticides) to pregnancy outcomes (specifically the risk of preterm delivery, miscarriage, small for gestational age [SGA] and sex ratio). If the males had used pesticides in the time period around conception, then the specific pesticides used were reported.

An increased risk of miscarriage was not associated with atrazine use as a crop herbicide (adjusted odds ratio [AOR] 1.5) or atrazine use as a yard herbicide (AOR = 1.2). An increased risk of SGA was not associated with atrazine or cyanazine use as a crop herbicide (AOR 0.5 and 0.8 respectively) or with atrazine use as a yard herbicide (AOR= 0.5). Sex ratio data were not separated out into exposures to specific chemicals but, since the AORs for proportion of male births for all farm activities involving chemicals ranged from 0.8 to 1.1, it is apparent that there was not an increased risk of alterations in sex ratio associated with use of any pesticide in males.

There was, however, an increased risk of preterm delivery associated with atrazine use by males around the time of conception. The AOR for use of atrazine as a crop herbicide was 2.4 (95% CI 0.8 to 7.0) and the AOR for use of atrazine as a yard herbicide was 4.9 (95% CI 1.6-15).

11.4 Summary and Conclusions

An association of exposure to atrazine-contaminated water and IUGR was seen, but because of the limitations of this study (*i.e.*, an ecologic study) the study authors conclude that the results are, "a preliminary finding that needs to be verified by more detailed epidemiologic studies." An association of atrazine use and birth defects in Minnesota communities was also seen, but the association was inconsistent. The inconsistencies in the data weaken the positive finding seen and the study authors do not dwell on the positive finding noted for atrazine. Male atrazine use around the time of conception failed to show an association for three of the four reproductive parameters examined (miscarriage, SGA and sex ratio). A positive association was seen for preterm delivery though. There is little biologic plausibility in associating a parameter such as preterm delivery with male chemical exposure. The effect of male chemical exposures on this endpoint has not been extensively studied and, as the study authors note: "Maternal characteristics, particularly reproductive and medical, are most strongly associated with preterm delivery."

The data provided by these three epidemiology studies do not provide clear evidence of an association between atrazine exposure and reproductive anomalies.

12. Background for Pregnancy, Pubertal and Prostatitis Papers

12.1 Role of Prolactin and LH in Pregnancy

Progesterone, acting at the uterus, is essential to maintain pregnancies in mammals. The major source of this vital progesterone in all mammals is the corpus luteum (CL). All mammals require progesterone throughout pregnancy from fertilization to parturition.

The sole source of progesterone during pregnancy in the rat is the CL. During early pregnancy (from implantation to approximately GD seven in the rat) the CL is maintained by prolactin derived from the anterior pituitary (Terkel, 1988). During mid-gestation (from GD 7 to 10) the CL is maintained by lutenizing hormone (LH) (Rothchild, 1981; Terkel, 1988). After GD 10, prolactin-like compounds produced by the placenta (*i.e*., placental lactogens) function to maintain the CL throughout the remainder of the pregnancy (Gibori, *et al.*, 1988; Linzner and Fisher, 1999).

The source of progesterone in pregnancy in humans is the CL for about the first six weeks of pregnancy. At approximately the sixth week of pregnancy, humans display a "luteal-placental shift" in which the placenta assumes the responsibility of progesterone secretion and the CL becomes quiescent (Stouffer, *et al.*, 1989).

Thus, all mammals require progesterone throughout pregnancy, but in the rat the only source of this progesterone is the CL whereas in the humans the CL is the initial source, and the placenta supplies progesterone for the greater part of the pregnancy. Maintenance of the CL in the rat is accomplished by prolactin, LH and placental lactogens. Pituitary-derived prolactin plays the primary role in maintaining the CL in early pregnancy; LH is primarily responsible for maintaining the CL in mid-gestation; and, placental lactogens maintain the CL during late gestation.

12.2 Role of Prolactin and LH in Pubertal Development

12.2.1 Female

Pubertal development in the female rat has been well-characterized (Ojeda, 1980; Ojeda, 1983). The onset of puberty in the female is a transitional period that culminates with the initiation of cyclic surges of luteinizing hormone (LH) from the pituitary that stimulate ovulation. Vaginal opening generally coincides with the first ovulation and occurs at 32 or 33 days of age in the female rat. The hormonal changes which induce the first ovulation are similar in many respects to the hormonal changes which induce all other ovulations in rodents. The sequence of hormonal changes preceding the first ovulation is as follows:

1. Serum estradiol levels increase followed by;

2. A dramatic increase (surge) in serum luteinizing hormone (LH);

3. Serum prolactin levels dramatically increase concomitant with the LH surge.

Exposure to atrazine has been shown to attenuate the proestrus LH and prolactin surges in Long-Evans and Sprague-Dawley rats. Since both of these hormones are important for normal pubertal development, it is reasonable to hypothesize that atrazine may affect the onset of puberty in the female rodent. Atrazine's attenuation of the proestrus LH surge is described in detail in Part B sections 9.2.7 and 9.2.8. Atrazine's attenuation of prolactin release is described below in section 13.1.

In addition, reports that atrazine can reduce hypothalamic norepinephrine concentrations (Cooper *et al.*, 1998) and that intravenous injections of GnRH restore the estrogen-induced secretion of LH in ovariectomized, atrazine-treated female rats (Cooper *et al.*, 2000) suggest that possible effects on neurotransmitters and their regulation of pituitary hormone synthesis/secretion could also alter the onset of puberty.

Thus, to examine the effects of atrazine on female pubertal development, a study was conducted using the "Research Protocol for the Assessment of Pubertal Development and Thyroid Function in Juvenile Female Rats" (U.S. EPA, 1998b; Goldman *et al.*, 2000).

12.2.2 Male

The onset of puberty in the male rat involves a complex interplay of several hormones including LH, FSH, testosterone and prolactin (Nazian and Mahesh, 1980; Piacsek and Goodspeed, 1978). It has been shown that an increased turnover rate in hypothalamic GnRH, NE and DA precedes the dramatic increase in testosterone (Matsumoto *et al.,* 1986) prior to the onset of puberty. LH stimulates testosterone secretion by the Leydig cells. At the same time, LH secretion varies only slightly as puberty approaches. However, there is an increased sensitivity of the testes to LH prior to puberty, due to other hormonal influences, such as increased prolactin secretion, that facilitate an upregulation of LH receptors (Kamberi *et al.*, 1980; Odell *et al.*, 1973; Vihko *et al.*, 1991). In contrast, there is a higher threshold for the gonadotropin/gonadal steroid feedback mechanism in the adult male (Gupta *et al.*, 1975; Nazian and Mahesh, 1980) as compared to the immature male, making the immature male more sensitive to the feedback of testosterone. As this feedback sensitivity decreases, the hypothalamic-pituitary unit becomes more effective at stimulating testicular development, because there is less inhibition of gonadotropins by testosterone.

Development of the size of the penis and cornification of the epithelium of the prepuce and preputial separation in immature rats are regulated by androgens (Marshall, 1966). A decrease in testosterone during the juvenile period can delay preputial separation (Lyons *et al.*, 1942) and reduce the size of the androgen-dependent tissues, such as the ventral prostate and seminal vesicles. Normally, testosterone levels rise gradually from PND 20 to 40, and abruptly double by PND 50 (Matsumoto *et al*., 1986; Monosson *et al*., 1999). Atrazine exposure has been shown to alter LH and prolactin secretion in female rats. An effect on LH and prolactin secretion in immature male rats, and thus on pubertal onset, may also be possible.

To examine the effects of atrazine on male pubertal development, a study was conducted using the "Research Protocol for the Assessment of Pubertal Development and Thyroid function in Juvenile Male Rats" (U.S. EPA, 1998b).

12.3 Role of Prolactin in Prostatitis

Hyperprolactinemia prior to puberty in male rats has been shown to lead to lateral prostate inflammation in young adult rats (Stoker *et al.*, 2000b). One possible cause of hyperprolactinemia in immature male rats is a deficiency in milk-derived prolactin. Milk-derived prolactin plays a critical role in the development of the tuberoinfundibular dopaminergic neurons (TIDA) of the hypothalamus of a developing rat (Shyr, *et al.*, 1986). The TIDA neurons function to inhibit prolactin secretion from the anterior pituitary. Organization and development of these neurons occurs mainly during the first postnatal week in the rat (Ojeda and McCann, 1974).

Thus, if developing rats do not receive a sufficient amount of prolactin from their mothers milk during the first week after birth, the TIDA neurons will not develop properly and may not be able to sufficiently provide an inhibitory check to prolactin secretion in the adult animal. The resultant hyperprolactinemia is associated with development of prostatitis in the adult.

13. Data

13.1 Atrazine Effects on Prolactin

Similar to the preovulatory LH surge that is described in Part B section 9.1.1. of this document, rodents also display a preovulatory prolactin surge (Blank, 1986). Studies demonstrating atrazine-associated effects on this prolactin surge are presented in Cooper, *et al.*, 2000 (other data from this publication relating to the site of action of atrazine-associated LH alterations are described in Part B section 9.3 of this document).

The prolactin studies described in Cooper *et al.*, 2000, use adult Long-Evans (LE) and Sprague-Dawley (SD) female rats which were ovariectomized (OVX) and given estrogen-containing implants. Three days later they were dosed by gavage with a single dose of 97.1% atrazine suspended in carboxymethylcellulose at dose levels of 0, 50, 100, 200, or 300 mg/kg (this protocol of OVX, estradiol implantation, and sacrifice three days later, is an established model of the LH and prolactin surges and was also used in the LH surge experiments described in Part B section 9.2.7). Separate groups of LE and SD females were OVX, implanted with estradiol pellets, and given daily doses at the same dose levels as the single-exposure animals for the three days leading up to sacrifice. Lastly, other LE and SD females were OVX, dosed daily for 21 days at dose levels of 0, 75, 150 or 300 mg/kg/day, implanted with estrogen pellets on day 21 and then sacrificed three days later.

Thus, atrazine exposures consisted of: single exposures; three-day exposures; and,21-day exposures. Following sacrifice, blood was collected and serum prolactin was measured.

A single exposure in the SD females resulted in no statistically-significant differences between controls and any of the dose groups. The LE rats showed statistically-significant decreases in serum prolactin levels in the high-dose group of 300 mg/kg only.

Three days of atrazine exposure resulted in statistically-significant attenuation of the prolactin surge in SD females at 300 mg/kg/day, but not at any other dose. The prolactin surge in LE rats under this exposure protocol was significantly attenuated at 100, 200 and 300 mg/kg/day and was delayed at 50 mg/kg/day.

The 21-day exposure resulted in significantly lower prolactin levels in both strain at the 150 and 300 mg/kg/day doses, but at 75 mg/kg/day prolactin levels

were not significantly different from control levels.

These data clearly demonstrate that atrazine has the ability to suppress estrogen-induced prolactin surges. Further data described in this paper demonstrate that, as was the case for the atrazine-associated LH surge attenuation, the effect of atrazine on prolactin secretion does not appear to be due to a direct effect on the pituitary. Rather, the hypothalamus appears to be the site of action for this effect of atrazine exposure.

13.2 Atrazine Effects on Pregnancy

13.2.1 Implantation and Early Pregnancy

Cummings *et al.*, 2000 (submitted) examined the effects of atrazine on implantation and early pregnancy in several strains of rats. Technical grade atrazine (ATR) of 97.1% purity was administered daily by gavage to rats during GD 1 to 8 (day 0 = sperm +). Dose levels included 0, 50, 100, and 200 mg/kg/day of ATR. Rats were divided into groups such that half were dosed at 2 p.m. (just prior to the diurnal prolactin surge of early pregnancy) and half were dosed at 2 a.m. (just prior to the nocturnal surge of prolactin). Within each time interval group, four strains of rats were each tested at each of the four dose levels listed above. Rat strains used were Holtzman, Fischer-344, Sprague-Dawley, and Long-Evans hooded. Clinical signs of toxicity consisted of decreased mean body weight at necropsy in the 200 mg/kg groups. Necropsies were performed on GD 9 of pregnancy. A small but significant decline in mean number of implantation sites was seen at 100 mg/kg in Fischer-344 rats (nocturnal dosing interval) and Sprague-Dawley rats (diurnal dosing interval), as well as at 200 mg/kg in Holtzman rats (nocturnal dosing). Holtzman rats alone showed both an increase in resorptions and a decrease in serum progesterone (at 200 mg/kg) as well as a decrease in serum LH at the same dose. Long-Evans and Fischer-344 rats also exhibited a decrease in serum LH in the 200 mg/kg dose group.

Summary/Conclusion

The LOAEL is 100 mg/kg for the effect on implantation and 200 mg/kg for all other parameters.

The NOEL is 50 mg/kg for the effect on implantation and 100 mg/kg for all other parameters.

13.2.2 Pregnancy Maintenance: Strain Comparisons of Sensitivity to Atrazine-Induced Pregnancy Loss in Rats

In a series of developmental toxicity studies (Narotsky *et al.*, 1999, Narotsky *et al.*, submitted) technical grade atrazine (97.1%) was administered by gavage, in 1% methylcellulose, to F344, Sprague-Dawley, and Long-Evans hooded rats at 0, 25, 50, 100, or 200 mg/kg/day on GD 6 to 10. This time frame was selected because it coincides with the LH-dependent period of pregnancy. In preliminary work, the authors identified this period of pregnancy as the most sensitive to the effects of atrazine. Using 200 mg/kg atrazine (by gavage), they found full-litter resorptions in 20 of 30 dams dosed from GD 6 to 10 while the same treatment was without effect in nine dams dosed from GD 11 to 15. Based on this information, the following study compared potential strain differences in response to atrazine. The dams were allowed to deliver and their litters were examined on PND's one and six. The F344 strain was the most sensitive to atrazine's effects on pregnancy maintenance; the Long-Evans strain was the least sensitive. In the F344 rats, maternal toxicity (weight loss, piloerection) and developmental toxicity (full-litter resorption, *i.e.*, pregnancy loss) were observed at $\geq$50 mg/kg. Among surviving litters, increased prenatal mortality was observed at 200 mg/kg, and parturition was delayed at 100 mg/kg. In Sprague-Dawley rats, similar effects were observed, albeit at different dose levels; maternal weight loss was noted at $\geq$25 mg/kg, full-litter resorption was observed only at 200 mg/kg, and delayed parturition was seen at $\geq$100 mg/kg. In contrast, the Long-Evans hooded strain showed maternal weight loss at $\geq$100 mg/kg and full-litter resorption at 200 mg/kg, but no effects on parturition.

Similar experiments conducted using the atrazine metabolites desethylatrazine, desisipropyl atrazine, diaminochlorotriazine and hydroxyatrazine, demonstrated that these metabolites were of equal or lesser potency than parent atrazine.

Summary/Conclusion

The maternal and developmental NOAELs and LOAELs for each strain are tabulated below.

	F344	Sprague-Dawley	Long -Evans
Maternal NOAEL	25 mg/kg	--	50 mg/kg
Maternal LOAEL	50 mg/kg	25 mg/kg	100 mg/kg
Developmental NOAEL	25 mg/kg	50 mg/kg	100 mg/kg
Developmental LOAEL	50 mg/kg	100 mg/kg	200 mg/kg

13.3 Atrazine Effects on Pubertal Development

13.3.1 Female

A recently completed study (Laws *et al.*, 2000, Laws *et al.* submitted) evaluated the effects of atrazine on pubertal development in the female Wistar rat. Atrazine (97.1%) was administered by oral gavage (in a suspension of 1% methyl cellulose) to 165 female Wistar rats, 15 or 30 rats/dose, at dose levels of 0, 12.5, 25, 50, 100 or 200 mg/kg/day, from PND 22 through 41. To evaluate the effects of lower body weight gain during treatment, a pair-fed group (n=15) was included where the food intake of each pair-fed rat was dependent upon the amount consumed by its respective mate in the ATR 200 mg/kg/day group. Half of the rats were killed on PND 41 and liver, kidney, adrenal, ovary, uterus and pituitary weights were collected. Estrous cyclicity was evaluated in the remaining females by monitoring changes in vaginal epithelial cells from vaginal opening through PND 70.

The mean body weights ± SEM for all treatment groups were equal on PND 22. Body weight on PND 41 was unaltered by 12.5, 25, 50 and 100 mg/kg/day, but was significantly reduced by 11% in the 200 mg/kg/day and 9% in the pair-fed groups. As compared with the control, the total gain in body weight during the 20-day treatment period was reduced to 17% and 15% in the ATR 200 mg/kg/day and pair-fed groups, respectively.

Vaginal opening was significantly delayed 2.3, 3.9 and 7.1 days following exposure to 50, 100 and 200 mg ATR/kg, respectively. In addition, vaginal opening did not occur in 18/31 females in the highest ATR dose group by the end of the dosing period. Body weight at the time of vaginal opening was significantly increased in the 50, 100 and 200 mg/kg/day groups as compared with the controls (as would be expected due to the increase in age at vaginal opening). However, no significant difference in the age or body weight at the time of vaginal opening was observed between the control and the pair-fed groups.

Irregular estrous cycles (*e.g*., increased number of days of diestrus) were observed between the time of vaginal opening and PND 41 in females exposed to 50 and 100 mg/kg/day, but returned to normal by the end of the 30-day exposure period. Once dosing was discontinued, vaginal opening occurred in all females in the 200 ATR group within four to five days. The estrous cycles in the ATR 200 females were irregular during the first 15-day interval following vaginal opening, but also returned to regular four to five day cycles by PND 70.

Summary/Conclusion

Atrazine exposure delayed vaginal opening and altered estrous cycles in female Wistar rats following oral exposure during PND 22 to 41. The LOAEL for vaginal opening is 50 mg/kg/day and the NOAEL is 25 mg/kg/day under the conditions of this protocol. The LOAEL for estrous cycle alteration is 50 mg/kg/day and the NOAEL is 25 mg/kg/day under the conditions of these assays. The effect on the estrous cycle is reversible as indicated by the fact that normal estrous cycles resumed in all females by the end of the 30-day post-exposure period. Data from this study are consistent with an effect on the central nervous system and subsequent alterations in hormonal control during pubertal development.

13.3.2 Male

Work from two separate laboratories has examined the effect of atrazine exposure on puberty onset in male rats. Data from the lab of Dr. Ralph Cooper (*et al.*, 2000) will be described first followed by data from the lab of Dr. Barry Zirkin (Trentacoste *et al.*, 2000).

Stoker *et al.*, 2000a, and Stoker *et al.*, submitted undertook a series of experiments using the weanling male Wistar rat. Animals were treated from PND day 23 to 53 with atrazine. Atrazine (97.1%) was administered by daily gavage to male Wistar rats of similar body weight at doses of 12.5, 25, 50, 100, 150 and 200 mg/kg/day. Six rats per dose were used at 12.5, 25, and 150 mg/kg/day and 20 to 24 per dose were used at 50, 100 and 200 mg/kg/day. An additional ten rats were pair-fed to match the food intake of the 200 mg/kg/day rats. Parameters measured were: body weights; prostate, seminal vesicle, epididymis and testes weights; preputial separation (PPS); and serum testosterone, estradiol, estrone, LH and prolactin levels. Organ weights and hormone measures were taken on day 53. Body weights and preputial separation were determined daily fro PND 23 to 53.

Body weights in the 200 mg/kg/day group were significantly decreased from day 43 to 53 compared to controls. There were no significant alterations in body weight in any other dose group. Testes weights (neither absolute nor relative to body weight) were not altered in any dose group. Absolute epidydimal and seminal vesicle weights were significantly decreased in the 200 mg/kg/day group and the pair-fed group. When adjusted for body weight, the seminal vesicles were still significantly reduced but the epididymis were not. Lateral prostate weights were not altered by atrazine treatment, but ventral prostate weights, both absolute and relative to body weight, were significantly decreased in all dose groups from 50 to 200 mg/kg/day. Serum hormone levels, for the most part, were not significantly altered by treatment with atrazine. There was, however, a statistically-significant increase in serum estrone and estradiol levels at 200 mg/kg/day compared to controls.

The major effect of atrazine on the male rats in this study was a delay in preputial separation. Preputial separation, which occurred on about day 42 in the controls in this study, was delayed by 2.3, 1.7, 1.7, 1.7 and 3 days in the 12.5, 50, 100, 150 and 200 mg/kg/day groups. The pair-fed animals displayed delays of two days compared to controls. A significant delay was not seen at the 25 mg/kg/day dose with the mean day of preputial separation being 43 days.

Trenatcoste, *et al.,* 2000 dosed male Sprague-Dawely rats by gavage from PND 22 to 47. Nine to twelve animals per dose level were dosed at 1, 2.5, 5, 10, 25, 50, 100 or 200 mg/kg/day of atrazine (96.1%). A separate study termed a "food deprivation study" was also conducted. In this study animals were dosed at 100 mg/kg/day and the amount of food consumed on a daily basis was measured. A second group of rats was vehicle-treated and fed the average daily intake of food consumed by the atrazine-treated group while a third group was vehicle-treated and fed *ad lib*. Parameters measured in both studies were body weights, serum and intratesticular (interstitial fluid) testosterone levels, serum LH levels, testes, epididymis, ventral prostate and seminal vesicle weights. Unlike the above described Stoker *et al.*, 2000a and Stoker *et al.*, submitted study, PPS was not measured in this study.

Body weights were significantly reduced in the 100 and 200 mg/kg/day dose groups compared to controls. Serum and intratesticular testosterone levels at 100 and 200 mg/kg/day were significantly reduced. Serum LH reduced 17 and 20% at he 100 and 200 mg/kg/day groups, respectively. Only the 200 mg/kg/day decrease in LH concentration was significant. Significant reductions in seminal vesicle and ventral prostate weight were seen at 100 and 200 mg/kg/day. Testes and epididymis weights were not significantly altered at any dose. Body weights, organ weights and hormone levels were not significantly altered at any dose from one to 50 mg/kg/day.

The food deprivation study also showed significant reductions in body weight, serum and interstitial testosterone, serum LH, ventral prostate weight, and seminal vesicle weight in rats treated with 100 mg/kg/day atrazine compared to vehicle-treated controls fed *ad lib*. The pair-fed, food deprived rats (the vehicle-treated rats fed the average daily intake of the 100 mg/kg/day rats) also showed significant decreases in serum and interstitial testosterone, serum LH, ventral prostate weight, and seminal vesicle weight compared to animals fed *ad lib*. The reductions in body weight, serum LH, and ventral prostate and seminal vesicle weights were almost identical between the 100 mg/kg/day and the pair-fed, food-deprived rats.

13.4 Atrazine Effects on Prostate

As described above, atrazine has been shown to depress the secretion of prolactin. Section 3.3. above describes the role of milk-derived prolactin in development of the TIDA neurons in the neonatal rat hypothalmus, and the resulting hyperprolactinemia followed by lateral prostatitis that is the consequence of incomplete development of these neurons. To summarize these points: without early lactational exposure to PRL, TIDA neuronal growth is impaired and elevated PRL levels are present in the prepubertal male. Hyperprolactinemia in the adult male rat has been implicated in the development of prostatitis.

Thus, early lactational exposure of dams to agents that suppress suckling-induced PRL release (possibly atrazine) could lead to a disruption in TIDA development in the suckling male offspring, followed by altered PRL regulation and subsequent hyperprolactinemia and prostatitis in these male offspring.

To test the hypothesis that atrazine exposure of dams during lactation could initiate the above-described sequence of events, Cooper *et al.*, 1999, measured suckling-induced PRL release in Wistar dams treated with atrazine (by gavage, twice daily on PND 1 to 4 at 0, 6.25, 12.5, 25, and 50 mg/kg) or the dopamine receptor agonist bromocriptine (BROM, s.c., twice daily at 0.052, 0.104, 0.208 and 0.417 mg/kg). BROM is known to suppress PRL release. Serum PRL was measured on PND 3 using a serial sampling technique and indwelling cardiac catheters.

A significant rise in serum PRL release was noted in all control females within 10 minutes of the initiation of suckling. Fifty mg/kg ATR inhibited suckling-induced PRL release in all females, whereas 25 and 12.5 mg/kg ATR inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of ATR was without effect. BROM also inhibited suckling-induced PRL release at the two highest doses.

To examine the effect of postnatal ATR and BROM on the incidence and severity of inflammation (INF) of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. While no effect was noted at 90 days of age, at 120 days both the incidence and severity of prostate inflammation was increased in those offspring of ATR-treated dams (25 and 50 mg/kg). The 12.5 mg/kg ATR and the two highest doses of BROM increased the incidence, but not severity, of prostatitis. Combined treatment of ovine prolactin (oPRL) and 25 or 50 mg/kg ATR on PND 1 to 4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in INF seen after ATR alone resulted from the suppression of PRL in the dam. Testing to determine whether or not there is a critical period for these effects revealed that the critical period for this effect is PND 1 to 9.

13.5 Summary/Conclusion

These data demonstrate that ATR suppresses suckling-induced PRL release and that this suppression results in an increase in lateral prostate inflammation in the offspring and that the critical period for this effect is PND 1 to 9.

13.5.1 Summary

The NOAELs for the above-described effects on pregnancy, pubertal onset and prostatitis are, for the most part, at or above 25 mg/kg/day. The exceptions are:

- A NOAEL for maternal effects in the pregnancy maintenance studies, in the SD rat strain only, was not found and the LOAEL is 25 mg/kg/day;

- The NOAEL for delay of pubertal onset in males is not clear as a significant delay was seen at 12.5 mg/kg/day, but not at the next highest dose of 25 mg/kg/day;

- The NOAEL for prostatitis is 12.5 mg/kg/day.